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THE EFFECT OF SALT CONCENTRATION ON THE FLUORESCENCE PARAMETERS OF ISOLATED CHLOROPLASTS

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SUMMARY

The previously reported effect of salt concentration on the fluorescence and other photochemical activities of Photosystem II is interpreted in terms of a change in the radiationless transition and the trapping probabilities This is confirmed by quantitative comparison of the fluorescence and the photochemical activity As a by-product of this analysis a method is devised to estimate the background fluorescence

We did not eliminate the possibility that the radiationless transition constant may include a contribution of energy transfer from Photosystem II to Photosystem I

INTRODUCTION

There are several reports in the literature on the effect of salt on Photosystem II reactions The effects caused on fluorescence induction reported by Murata [1, 2] and by Homman [3] are particularly noteworthy In general, both the quantum yield of fluorescence [1–3] and of Photosystem II photochemistry [4–6] increase by adding salt into the medium

The reason for this effect is not well understood Most authors speak loosely about the change of light distribution between Photosystem I and Photosystem II This explanation is very unlikely physically It is hard to imagine how chlorophyll molecules change their location and move in an ordered manner between two separate Photosystem I and Photosystem II entities

The explanation becomes easy if one interprets the effect in terms of a change in the quantum yields of the primary photophysical events in Photosystem II The light distribution remains essentially the same, but the quantum yields of both fluorescence and photochemistry change As we shall show the reason for these changes comes from a drastic change in the radiationless transition constant Also, the trapping constant is probably changed A quantitative correlation between the photochemical yield and fluorescence yield changes, calculated on this basis, is quite satisfactory

Abbreviation DCIP, dichlorophenolindophenol

Another possible suggestion is of a dynamic spill-over of excitation from Photosystem II to Photosystem I, controlled by the state of the Photosystem II reaction centers, which is inhibited in the presence of salt [1, cf also refs 7 and 8*] This possibility may be included in the framework of the same theory mentioned above, except that the interpretation for the radiationless transition constant is changed and must also include the rate constant for energy transfer from Photosystem II to Photosystem I Thus, although the term radiationless transition will be used in the following text the possibility of energy transfer to Photosystem I is not eliminated Its significance must be proved or eliminated by other experiments which we pursue now

THEORY

The typical fluorescence rise (Fig 1) observed in isolated chloroplasts upon irradiation probably reflects the transition from a state of maximal trapping to a state of vanishing trapping [9] By trapping we mean the utilization of excitation energy in the reaction center for the primary photoreactions If we denote by k_F, k_H and k_T the rate constants for the fluorescence, radiationless transition** and trapping, respectively, we can describe the fluorescence yields [9, 10] as the following ratio of rate constants

$$\phi_{Fo} = \frac{k_F}{k_F + k_H + k_T} \tag{1}$$

$$\phi_{Fm} = \frac{k_F}{k_F + k_H} \tag{2}$$

ϕ_{Fo}, ϕ_{Fm} = fluorescences yields for maximal trapping and vanishing trapping, respectively

In this description k_T depends on the number of trapping centers Since we are only interested in this article in the initial and final states there is no significance in the type of model used for the photosynthetic unit, whether independent or statistical [10–12] For the independent unit model k_T is a constant of the unit For the statistical model k_T is a function of the initial concentration of the traps

In this theoretical framework the yield of trapping for the state of maximal trapping will be given by

$$\phi_T = \frac{k_T}{k_F + k_H + k_T} \tag{3}$$

From Eqns 1 and 2 ϕ_T is also given by

$$\phi_T = 1 - \frac{\phi_{Fo}}{\phi_{Fm}} \tag{4}$$

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* The work of Briantais et al , although different in detail is very similar in essence to the present work It was brought to our attention after this work was completed

** In this article k_H includes also intersystem crossing to the triplet manifold (cf later)

All the above formulae are relevant to Photosystem II For photoreactions limited by Photosystem II or which are induced by Photosystem II alone the maximal quantum yield of a photoreaction in steady light will be given by

$$\phi_{PC} = \alpha \quad \phi_T = \alpha \quad \left(1 - \frac{\phi_{Fo}}{\phi_{Fm}}\right) \tag{5}$$

where α is the fraction of the absorbed light channelled into Photosystem II For a set of conditions that do not change α, ϕ_{PC} is expected to be proportional to

$$\left(1 - \frac{\phi_{Fo}}{\phi_{Fm}}\right)$$

During the course of many years of work we noticed quite considerable variations in the ratio ϕ_{Fo}/ϕ_{Fm} for different samples This ratio could not always be correlated to ϕ_{PC} In order to develop a systematic approach we must assume that part of the fluorescence must be subtracted as a background contributed by irrelevant sources other than active Photosystem II units The estimation of background fluorescence is somewhat controversial Thus Clayton [13] and Lavorel [14] estimate a relatively large background contribution to ϕ_{F_o} while Tumerman [15], from fluorescence life-time studies deduces that most ϕ_{F_o} is a relevant emission from Photosystem II In bacterial systems Borisov and Godik [16] estimated (by the fluorescence lifetime technique) that a considerable fraction of the fluorescence is background emission The best way to proceed is to assume that in different circumstances the contribution of background fluorescence is different

Considering the background fluorescence Eqn 4 will be modified by subtracting a background fluorescence ϕ_{Fb}

$$\phi_T = 1 - \frac{\phi_{Fo} - \phi_{Fb}}{\phi_{Fm} - \phi_{Fb}} \tag{6}$$

RESULTS

Fig 1 shows the salt effect on the fluorescence induction curve, at high salt concentration Relative to low salt, there is only a slight increase in the initial yield of the fluorescence, but a remarkable increase in the final yield This confirms the work of Homman [3]

Fig 2 shows how the initial and final fluorescence yield depend on the salt concentration The final fluorescence yields may increase by as much as a factor of 2 4 in the transition from 0 01 M to 0 2 M NaCl The initial fluorescence apparently follows the same trend but its change is relatively small ($\approx$ 10–20 %) At higher concentrations ϕ_{Fm} is optimal in the range of 0 1–0 3 M NaCl

Fig 2 represents an experiment in which there is a strong salt effect on the fluorescence, but relatively little or no effect on the oxygen evolution rate in limiting light (cf Table 1) This experiment shows that we ought not to explain the salt effect in terms of the light distribution into Photosystem II since the light distribution factor should act on both initial fluorescence, final fluorescence and the quantum yield of photochemical reactions in the same quantitative way This fact has puzzled previous investigators [3]

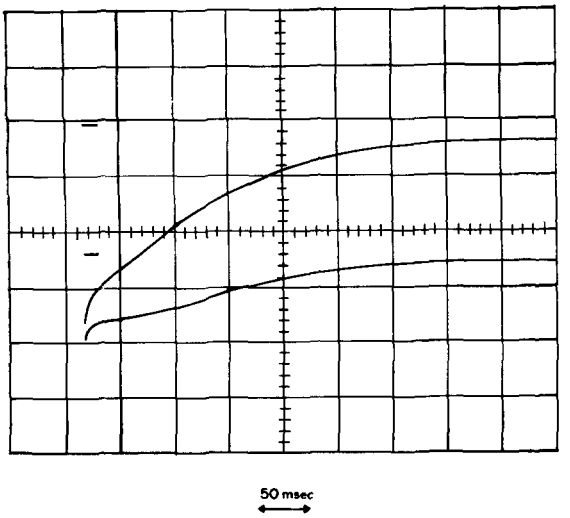

Fig 1 Fluorescence induction curves obtained in a medium containing 0 01 M NaCl (bottom trace) and 0 1 M NaCl (upper trace) The medium contained also 0 005 M tricine, pH 7 5 Chloroplasts (from pea) were prepared as described before [15] The fluorescence measurement was done by a standard procedure [7] after a relaxation time of 2 min in the dark following a prior short exposure The exciting light was isolated by a 600 nm filter (band width 15 nm) Light intensity $\approx$ 10 nEinstein cm^{-2} s^{-1} and the fluorescence light was isolated by a sharp interference filter peaked at 685 nm (band width 3 nm) Chlorophyll concentration 10 μg/ml

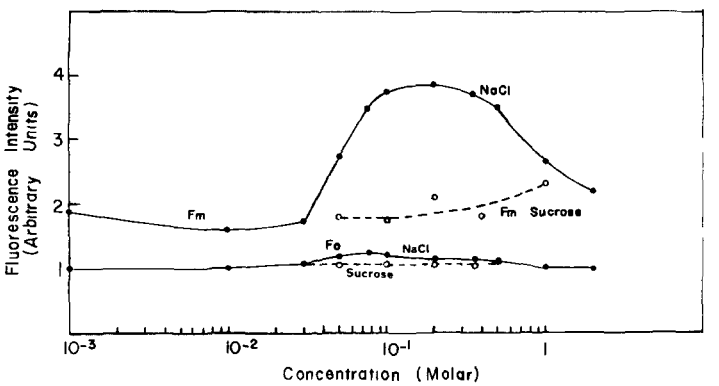

Fig 2 The effect of various NaCl concentrations on the fluorescence induction parameters, *Fo* and *Fm* (initial and final values, respectively) The fluorescence values are expressed in arbitrary units and a value *Fo* = 1 was chosen for 0 01 M NaCl - - -, effect of sucrose added on top of the initial NaCl concentration of 0 03 M This experiment was done on a completely different sample grown in different conditions than the experiment of Fig 1 Other conditions are similar

There are other experiments which did show an increase of photochemistry quantum yield parallel to the fluorescence However, the change in the photochemistry is always much less compared to the change in the maximal fluorescence

In order to analyze the data in terms of changes in the fluorescence yield parameters we checked that the low value of final fluorescence at low salt is not caused

TABLE I

EFFECT OF SALT ON O_2 EVOLUTION IN PRESENCE OF POTASSIUM FERRICYANIDE

The same preparation as that in the legend to Fig 2 was used, under limiting light conditions O_2 evolution was monitored by YSI O_2 electrode Reaction mixture contained 10^{-4} M $K_3Fe(CN)_6$ The light intensity was ≈ 1 nEinstein cm^{-2} s^{-1} The saturation rate in strong white light was approximately 10 times higher than the rate at limiting light conditions

NaCl concentration (M)	O_2 evolution rate (arbitrary units)
0 01	23 ± 3
0 1	24 ± 3

by an approach to an equilibrium between the light reaction (which tends to increase the fluorescence) and the reverse dark reaction (which tends to decrease the fluorescence) This possibility was eliminated by the observation that reducing the light intensity by a factor of 2 and 3 did not cause any essential differences in the result

The salt effect is certainly not osmotic, since it is not shown by adding sucrose (Fig 2), except perhaps at a very high concentration, where the fluorescence yield drops

ANALYSIS OF THE RESULTS

As already mentioned the explanation of the salt effect in terms of changes in α, the light distribution of Photosystem II, fails after quantitative examination We now check the explanation in terms of a change in ϕ_T, according to Eqn 4

Analysis of Homman's results (ref 3 Fig 1)

From the ratio of the values of ϕ_{Fo} and ϕ_{Fm} we calculated ϕ_T for low and high salt concentrations (In this experiment the salt effect is achieved by adding a small amount (3 3 mM) of $MgCl_2$) The ratio of quantum yields obtained by this method was 1 1 (high salt/low salt) From the induction curves plotted on the same scale we found that the high salt curve is faster by a factor of $1\ 15 \pm 0\ 05$ Assuming that the induction process reflects a reduction of the same pool of electron carriers in both cases [7], the ratio of the induction times is equal to the reciprocal ratio of the quantum yields* Therefore there is an agreement between the values of the fluorescence parameters and the fluorescence kinetics There is no need to assume any background fluorescence This experiment is a nice demonstration that the salt effect affects ϕ_T rather than the distribution of light into Photosystem II

* The standard formula for the induction time [7], using the present notation, is

$$n = \phi_T \alpha\, I \int_0^{\infty} \frac{\phi_F - \phi_{Fo}}{\phi_{Fm} - \phi_{Fo}}\, dt$$

(where n is the number of electrons transferred, I is the absorbed light intensity) The integral defines the time of induction $\bar{t}$ Hence

$$\frac{\phi_{T1}}{\phi_{T2}} = \frac{\bar{t}_2}{\bar{t}_1}$$

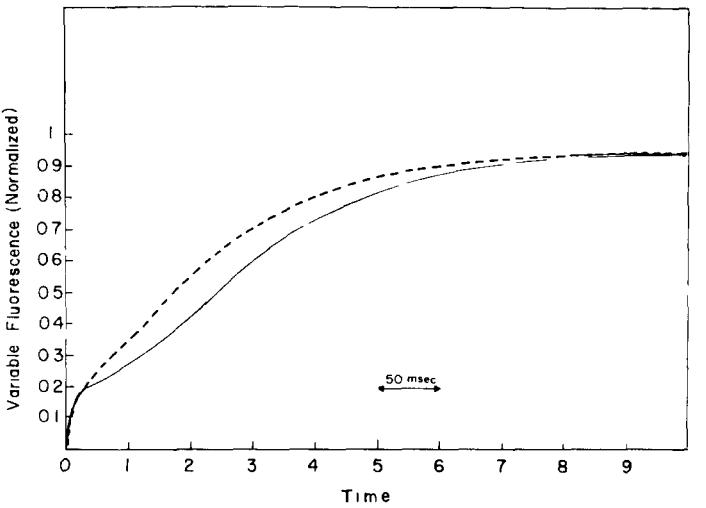

Fig 3 Fluorescence induction curves of Fig 1 normalized such that the variable fluorescence value is 1 - —, 0 01 M NaCl - - -, 0 1 M NaCl

Analysis of Fig 1

Analysis of this experiment shows the need to subtract an appropriate value of a background fluorescence, according to Eqn 6 In Table II we brought the results of computations based on Eqn 6 We compared the ratios of ϕ_T for low and high salt media calculated from Eqn 6, assuming different values of ϕ_{Fb}, to ratios of ϕ_T calculated from the induction curves (as in the analysis for ref 3) The normalized induction curves are shown in Fig 3, the high salt curve is faster by a factor of 1 2±0 05 It is seen that an agreement between the measured and computed ratios of ϕ_T is obtained if we assume a background fluorescence in the range 0 3–0 6 (relative to a value 1 of ϕ_{Fo} for the low salt medium) It can be seen also that this method for estimating ϕ_{Fb} is not very sensitive and can give only crude estimates, depending on the accuracy in ϕ_T

To show that the quantum yield of the fluorescence induction process is also reflected in the external electron transfer we also measured the reduction of DCIP in limiting light by standard methods [18] The same ratio of quantum yields was also obtained in this method for the two salt concentrations

Analysis of Fig 2, and Table I

In these experiments there was a relatively big salt effect on the fluorescence parameters, but almost no change on the quantum yield, Table I In these experiments we measured the quantum yield by measuring the oxygen evolution in limiting light Within experimental error we may assume that ϕ_T = a const Therefore, $1-\phi_{Fo}/\phi_{Fm}$ must be approximately constant Either ϕ_{Fo}/ϕ_{Fm} is constant or else it must be very small compared to 1 Both conclusions are not compatible with the calculated values for ϕ_T of 0 63 and 0 31 for low and high salt, respectively Hence we must subtract a certain value ϕ_{Fb} as a background, according to Eqn 6

Table II shows the computed values of ϕ_T from different assumptions about the background fluorescence, for 0 01 M and 0 1 M salt concentration It is clear that for

TABLE II

EXPERIMENTAL AGREEMENT BETWEEN ϕ_T RATIOS COMPUTED FROM FLUORESCENCE YIELDS (EQNS 4–6) AND OBTAINED BY DIRECT EXPERIMENT

Dashed and undashed values refer to two different media, the dashed values refer to higher fluorescence values (high salt) Fluorescence values are expressed in relative units ϕ_{Fo} is given the value of 1 for all experiments

Expt	ϕ_{Fo}	ϕ_{Fm}	ϕ'_{Fo}	ϕ'_{Fm}	ϕ_{Fb}	Computed ratio ϕ_T/ϕ_T (Eqn 6)	Measured ratio ϕ_T/ϕ_T
Ref 3 Fig 1	1	4 4	1 1	8	0	1 1	1 1±0 05
Fig 1	1	2	1 14	3 5	0	1 35	
					0 2	1 29	
					0 3	1 25	1 2±0 05
					0 4	1 22	Agreement within
					0 5	1 18	experimental error
					0 6	1 14	
					0 8	1 05	
Fig 2 and Table I (0 01 M and 0 1 M NaCl)	1	1 6	1 2	3 5	0	1 75	
					0 2	1 62	
					0 4	1 48	
					0 6	1 32	
					0 7	1 23	1±0 25
					0 8	1 14	Agreement within
					0 9	1 03	experimental error
					1	0 94	

a value ϕ_{Fb} equal or greater than about 70 % of the ϕ_{Fo} value at 0 01 M the ratio between the computed ϕ_T values lies in the range of experimental accuracy (Table I) This is an example where the background fluorescence is very considerable

Rationale for the salt effect Computation of changes in k_H and k_t

In order to see the reason behind the salt effect we must formulate what are the affected parameters Since the quantum yield of fluorescence is always low [19] (in the order of 1–2 %) we can always neglect k_F compared to k_H and k_T We obtain the following simplified expressions

$$\phi_{Fo} \approx \frac{k_F}{k_H + k_T} \qquad \phi_{Fm} \approx \frac{k_F}{k_H} \tag{7}$$

We confidently assume that k_F is not affected by the medium* Since the biggest effect of the medium is on ϕ_{Fm} it is certainly concluded that k_H decreases considerably at high salt compared to low salt This will affect ϕ_{Fo} in the same direction as ϕ_{Fm}, but to a much lesser degree due to the factor k_T (since $k_H < k_T$)

If we compare two media between which there is a maximal effect it is easy to show from Eqn 7 that

* k_F is related to the absorption coefficient through the Einstein relations This factor is not expected to change as much as there is no essential change in the molecular structure From the fact that the absorption and emission spectra remain the same we must conclude that k_F is not changed

TABLE III

CALCULATION OF CHANGES IN k_H THE RADIATIONLESS TRANSITION CONSTANT AND k_T, THE TRAPPING CONSTANT

For reasonable assumption on the background fluorescence cf Table II

Expt	Background fluorescence	k_H/k_H	k'_T/k_T
Ref 3 Fig 1	0 0	0 53	1 01
Fig 1	0 3	0 53	1 05
	0 4	0 52	0 99
	0 5	0 50	0 92
	0 6	0 48	0 84
Figs 2 and 3	0 7	0 32	0 74
	0 8	0 30	0 57
	0 9	0 27	0 37
	1 0	0 24	0 0

Cf explanations to Table II

$$\frac{k'_H}{k_H} = \frac{Fm}{Fm'}$$

$$\frac{k'_T}{k_T} = \frac{\frac{1}{Fo'} - \frac{1}{Fm'}}{\frac{1}{Fo} - \frac{1}{Fm}} \qquad (8)$$

where Fo and Fm are the relative values of the fluorescence parameters, expressed arbitrarily in any convenient unit, the dashed and undashed values refer to the two media, respectively The results of the computations based on Eqn 8 are summarized in Table II Evidently both k_H and k_T change k_H is decreased by a factor of 2–3 in the transition from low to high salt The calculation of k_T is much less certain, depending strongly on the assumption made for the background fluorescence Two experiments indicate no change or only a very small decrease ($\approx 20\,\%$) by the salt addition One experiment indicates a more considerable decrease in k_T Much more accurate experiments will be needed to estimate k_T changes with any certainty The present results are only indicative

It is known that in vitro chlorophyll shows a decrease in its fluorescence as a function of its concentration (concentration quenching) [20–22] which is a manifestation of increasing k_H Although the (microscopic) chlorophyll concentration in vivo is surely very high, the pigments are relatively protected from those interactions which cause too high a value of k_H This protection may be due to a more ordered state of the pigment aggregate in vivo which limits a closer approach of any two pigments molecules and thus eliminates in part this interaction The ordered state is controlled presumably by the membrane macromolecules The salt effect is presumably an electrostatic effect on the membrane polyelectrolytes which in turn causes conformational changes and may change typical distances in the membrane sites for chlorophyll There is evidence to show drastic membrane changes specific to salt interactions [23–25] which are not osmotic [24]

The salt effect does not only change k_H but also the interaction between different photosynthetic units According to Marsho and Kok [6] at the low salt medium the energy transfer characteristics indicate independent photosynthetic units, while at high salt there is a probability ($\approx$ 0 5) of energy transfer between a closed unit to an adjacent unit [26]

To summarize, high salt medium tends to decrease k_H and perhaps also k_T This causes big changes in the maximal fluorescence while affecting the initial fluorescence only slightly The initial fluorescence change may also be masked by a background contribution The effect on k_H (and k_Γ) causes increased trapping efficiency and hence a higher Photosystem II activity However, the effect on photochemical activities may not sometimes be noticed if the efficiency was already close to a maximum at low salt medium (Figs 2 and 3) The enhanced Photosystem II activity may explain also the appearance of Emerson enhancement at high salt [5] and the changes in the redox state of intermediate electron carriers between the two photosystems caused by salt in weak light [6]

We must now consider what is the contribution of the energy transfer constant k_t in k_H As stated in the introduction we include in k_H all other contributions besides fluorescence and photochemistry to the loss of excitation from Photosystem II At this stage we will make only a preliminary consideration

The most convincing evidence in favor of energy transfer is that of Murata [1] who showed that the addition of Mg^{2+} salts increases the quantum yield of Photosystem II DCIP reduction while decreasing the quantum yield of Photosystem I $NADP^+$ reduction by $DCIPH_2$ It is not an absolute evidence, since no one showed that the loss in Photosystem II makes for the gain in Photosystem I It is possible that independently of each other the trapping efficiency of one system increases while the other decreases Also, under the limiting light conditions used in Murata's work [1], one can conclude that the trapping centers of Photosystem II are opened while the energy transfer goes on The yield of the (supposed) energy transfer is quite high, even when the reaction centers are opened and supposed to trap the excitation efficiently (It is at least 25 % of the trapped excitation in Photosystem II, cf ref 1, Figs 5–7, judged from the Mg^{2+} effect) One must wonder how such energy transfer may be so efficient compared to the trapping efficiency, when we know that energy transfer between Photosystem II units themselves is not so efficient [27], and that Photosystem I and Photosystem II form separate entities [28]

Different possibilities may be sought One possibility is that Photosystem I reactions utilize triplet excitons k_H includes intersystem-crossing into the triplet manifold Depending on life-time and the interactions between the pigments, the triplet may have a high probability to be trapped at the Photosystem I center In this way we keep both the idea of energy transfer and the meaning of k_H as a radiationless transition constant We are now investigating this and other possibilities

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